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USE OF THE VINYLOXYCARBONYL GROUP FOR AMINO PROTECTION IN PEPTIDE SYNTHESIS†

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In this communication we introduce a promising masking agent -- the vinyloxycarbonyl or VOC group -- for the protection of amino functions in peptide chemistry.

Amino acids are easily converted to their N-VOC derivatives (1) in dilute aqueous base using the known vinyl chloroformate[1] in dioxane as the acylating agent. The pH of the reaction medium is controlled (9-10) with a pH stat or by using an MgO suspension as the OH^- source, two procedures[2] widely utilized for the preparation of BOC and Z amino acids.[3] Some of the VOC amino acids made are listed in Table I.[4] Like BOC-AA's, the acids (1) are often oils but are easily characterized as the crystalline dicyclohexylammonium (DCHA) salts (2) from which 1 can be regenerated by extraction from carefully acidified solutions. Some VOC peptides (3) made

Table I. Representative N-VOC Amino Acids and Peptides[4]

VOC-Gly-OH	mp 95-95.5°	VOC-Gly-Gly-OEt	mp 115-115.5°
VOC-L-Phe-OH	56-58°	VOC-Gly-Gly-Gly-OEt	173-175°
VOC-L-Ala-OH DCHA	152.5-153°	VOC-L-Phe-Gly-OEt	127.5-129°
VOC-L-Val-OH DCHA	160-161.5°	VOC-L-Phe-L-Leu-OMe	107.5-108.5°
VOC-L-Pro-OH	93-94.5°	VOC-L-Asn-L-Leu-OMe	167-168.5°
VOC-L-Asn-OH	157.5-158.5°	VOC-L-Pro-Gly-OH DCHA	157-160°
VOC-L-Hypro-OH DCHA	179.5-181.5°	VOC-L-Glu(γOMe)-Gly-OEt	88-89°
VOC-L-Glu(γOMe)-OH DCHA	155-156°	VOC-L-Asn-Gly-Gly-OEt	190.5-192°
VOC-L-Ser(O-tBu)-OH	97-97.5°	VOC-L-Phe-L-Leu-O-Phenacyl	151-152°
VOC-L-Lys(εBOC)-OtBu	62-63.5°	VOC-L-Pro-L-Val-L-Asn-L-Leu-OtBu	216° dec
VOC-L-Lys(εBOC)-OH DCHA	152-154°	VOC-L-Ser(OtBu)-L-Phe-L-Leu-O-Phenacyl	161-163°

†This communication and the three closely related papers which follow are dedicated by the senior author to his mentor, Professor R. B. Woodward, on the occasion of his sixtieth birthday. Also acknowledged with special gratitude is Dr. Woodward's early encouragement of the research described here.

from the free acids (1) or directly from the salts (2) using N-ethyl-5-phenylisoxazolium-3'-sulfonate, dicyclohexylcarbodiimide, or an active ester as the coupling agent[5] are also included in Table I. Crystalline compounds (1-3) are stable and can be stored indefinitely. Yields in the synthesis of VOC-AA's and peptides are similar to those found in the analogous chemistry of BOC peptides.

The exceptional reactivity of the VOC-amide C=C bond toward electrophiles provided the key to the three procedures which have been developed for removal of the N-VOC moiety:

Acid-Induced Deprotection: Treatment of VOC-Gly_2-OEt with anhydrous HCl in dioxane (standard N-BOC scission medium) yields the unstable adduct (4, mp 118-120°, NMR: MeCHCl- at δ 1.77 [d] and 6.56 [q] in CD_3CN) which, when warmed in ethanol, affords the dipeptide salt (6) along with the volatile byproducts, CO_2 and acetal.

$$H_2C{=}CHOC(=O)\text{-}Gly_2\text{-}OEt \xrightarrow[\text{r.t.}]{\text{HCl in dioxane}} \underset{4}{MeCHClOC(=O)\text{-}Gly_2\text{-}OEt} \xrightarrow[\text{30 min}]{\text{EtOH, 50°}} [MeCH(OEt)OC(=O)\text{-}Gly_2\text{-}OEt] \underset{5}{} \rightarrow\rightarrow \underset{6}{HCl\cdot H\text{-}Gly_2\text{-}OEt} + CO_2 + MeCH(OEt)_2$$

In the sequence, 4→6, the rate determining step is formation of the acylal (5) by a simple S_N1 substitution. This intermediate is so reactive that no acid beyond that produced in the cleavage is required to complete its conversion to 6. The complete unmasking was performed in 97% overall yield by removal of the excess HCl-dioxane in vacuo followed by addition of ethanol to the residue. Similarly the glutamate peptide (7) has been unblocked in 95% yield[6] by bubbling HCl through a solution of 7 in CH_2Cl_2 followed by ethanol treatment.

$$\underset{7}{\text{VOC-L-Glu(}\gamma\text{OMe)-Gly-OEt}} \xrightarrow[\text{r.t.}]{\text{HCl in } CH_2Cl_2} \xrightarrow{\text{evap.}} \xrightarrow{\text{warm EtOH}} HCl\cdot H\text{-L-Glu(}\gamma\text{OMe)-Gly-OEt}^{7}$$

During ethanolysis, the acidity of the medium is low enough to avoid transesterification of the glutamyl γ-methyl ester group. Other acid-induced scissions including one-step processes and reactions under the more stringent conditions required to unmask Z-peptides are depicted below:

$$\text{VOC-L-Phe-L-Leu-OMe} \xrightarrow{\text{HBr in HOAc}} \underset{\text{(mp 123-126°)}}{HBr\cdot H\text{-L-Phe-L-Leu-OMe}} \quad 93\%\ \text{yield}$$
(HCl salt in 95% yield by HCl/CH_2Cl_2 process)

$$\text{VOC-Gly-Gly-Gly-OEt} \xrightarrow{\text{HCl in HOAc}} HCl\cdot H\text{-Gly-Gly-Gly-OEt} \quad 93\%\ \text{yield}$$
(96% yield with HCl in absolute ethanol)

$$\text{VOC-L-Asn-L-Leu-OMe} \xrightarrow[\text{through } CH_2Cl_2]{\text{bubble HBr}} \xrightarrow[\text{b) MeOH}]{\text{a) evap.}} \underset{\text{(mp 124-126°)}}{HBr\cdot H\text{-L-Asn-L-Leu-OMe}} \quad 94\%\ \text{yield}$$

Deprotection by Bromination-Alcoholysis: Titration of the highly activated vinyl urethan group of simple VOC-peptides (3) with one equivalent of standardized Br_2 in CH_2Cl_2 to give the adduct (8) occurs so cleanly and rapidly that the process may be used as a quantitative assay for 3 - a fugitive yellow color appears at the end point.

$$\underset{3}{H_2C{=}CHOC\text{-}NHR} \xrightarrow[CH_2Cl_2]{Br_2\ in} \underset{8}{BrCH_2CHBrOC\text{-}NHR} \xrightarrow{HOR'} \underset{9}{HBr{\cdot}H_2NR} + CO_2 + BrCH_2CH(OR')_2$$

Though unstable, 8 can sometimes be isolated, e.g. 8[R=H]: mp 122.5-123°, NMR: δ 3.98 [d,2], 5.2-6.2 [s,2], 6.77 [t,1], from VOC-NH_2 (mp 54.5-56.5°). The adducts (8) readily cleave to the desired HBr salts (9) when methanol or ethanol is added to the reaction mixture. Because of the increased susceptibility of 8 (vs. 4) to S_N1 substitution, it is often unnecessary to heat the mixture to complete the unblocking. Other VOC unmaskings by this method include:

VOC-L-Phe-Gly-OEt $\xrightarrow[CH_2Cl_2]{Br_2\ in}$ $\xrightarrow{add\ EtOH}$ HBr·H-L-Phe-Gly-OEt[8] 93% yield

VOC-Gly-Gly-Gly-OEt (10) $\xrightarrow[to\ peptide\ in\ EtOH]{add\ Br_2\ in\ CH_2Cl_2}$ HBr·H-Gly-Gly-Gly-OEt 97% yield

VOC-L-Pro-L-Val-L-Asn-L-Leu-OtBu $\xrightarrow[to\ peptide\ in\ MeOH]{add\ Br_2\ in\ CH_2Cl_2}$ HBr·H-L-Pro-L-Val-L-Asn-L-Leu-OtBu (anal. pure: 98% yield; mp 172° d)

Note the lack of complications from electrophilic aromatic substitution of phenylalanine, the successful use of a hydroxylic solvent during bromination, and the inertness of asparagine and the t-butyl ester unit. Only Trp, Tyr, Cys, and Met are likely candidates for side reactions with Br_2 under the experimental conditions. This procedure was tested partly in the hope that it would allow the use of the BOC group to mask secondary functions in peptide synthesis. Most agents now used for this purpose (benzyl, tosyl, etc.) are sometimes so stable they can only be detached under conditions in which the product peptide is itself partially dismembered. Two experiments confirm this hope. First, selective VOC removal from VOC-L-Lys(εBOC)-OtBu to give HBr·H-L-Lys(εBOC)-OtBu (mp 91-92.5°) was accomplished in 85% yield. In an even more sensitive test, (10) was cleaved in the presence of an equal amount of BOC-[1-^{14}C]Gly-Gly-Gly-OEt. With 0.03 eq Bu_3N present to neutralize any strong acid impurity in the Br_2, the BOC peptide was reisolated and the HBr·H-Gly_3-OEt product contained only 0.4% of radioactive material.

Deprotection with Hg^{2+}: In a last electrophile-induced[9] scission, VOC-L-Phe-OH was unmasked with $Hg(OAc)_2$ in 9:1 HOAc-H_2O at 25° in 97±2% yield (isotope dilution assay). Since Hg^{2+} separation from small peptide esters is impractical, it is expected that this mild unblocking

method will only be useful for large peptides where chromatography is routinely used.

Carboxyl Protecting Agents: While the tertiary amide, VOC-L-Pro-Gly-OEt, cleanly affords VOC-L-Pro-Gly-OH on reaction with 1.2 eq 0.5 N NaOH in MeOH-water, secondary VOC-amides only partly survive such treatment. In those situations where carboxyl unmasking prior to VOC removal is desired, the use of phenacyl esters for carboxyl protection[10] is especially recommended:

$$\text{VOC-L-Phe-L-Leu-O-Phenacyl} \xrightarrow[CH_2Cl_2]{\text{HCl in}} \xrightarrow[\text{MeOH}]{\text{warm}} \underset{\text{(mp 182-185°)}}{\text{HCl·H-L-Phe-L-Leu-O-Phenacyl}} \quad 89\%\ \text{yield}$$

$$\text{VOC-L-Ser(OtBu)-L-Phe-L-Leu-O-Phenacyl} \xrightarrow[\text{HOAc at 25°}]{\text{Zn in aq}} \underset{\text{(mp 183-184.5°)}}{\text{VOC-L-Ser(OtBu)-L-Phe-L-Leu-OH}} \quad 91\%\ \text{yield}$$

The use of VOC-Cl as a reagent for hydroxyl protection and for the N-dealkylation of tertiary amines is outlined in the accompanying communications.[11]

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Footnotes and References

1) F. E. Küng, U.S. Patent 2,377,085, May 29, 1945; L.-H. Lee, J. Org. Chem., 30, 3943 (1965). Phosgene and ethylene glycol are combined to yield $(CH_2OCOCl)_2$ which is converted to VOC-Cl when passed through a hot tube. Since the reagents are cheap and the complete synthesis can be performed as a single flow process without solvent, VOC-Cl is potentially quite inexpensive on an industrial scale.

2) E. Schnabel, Ann. Chem., 702, 188 (1967); R. Schwyzer, P. Sieber, and H. Kappeler, Helv. Chim. Acta, 42, 2622 (1959).

3) Standard abbreviations for amino acids (AA's) and protecting groups are used: BOC is t-butoxycarbonyl, Z is carbobenzoxy.

4) Satisfactory combustion analyses and corroborative spectroscopic data have been obtained for all crystalline new compounds (identified by inclusion of a mp where introduced). NMR vinyl absorptions at δ 4.4-4.5, 4.7-4.8, and 7.1-7.2 are valuable in the quantitative and qualitative assay of 1-3. Multiprotected AA's in Table I were made by standard methods.

5) R. B. Woodward, H. Mayer, and R. A. Olofson, Tetrahedron, Suppl. 8, Pt. I, 321 (1967); J. Amer. Chem. Soc., 83, 1010 (1961); J. C. Sheehan and G. P. Hess, ibid., 77, 1067 (1955); M. Bodanszky and V. du Vigneaud, ibid., 81, 5688 (1959); D. S. Kemp and R. B. Woodward, Tetrahedron, 21, 3019 (1965).

6) Yields given are isolated yields of crystalline product and thus minimum values. Since crude peptide ester hydrohalides (no matter how generated) are often hygroscopic, difficult to crystallize materials, high manipulative loss on purification diminishes the utility of yield as a measure of the value of a deprotection method. In actual peptide syntheses, crude product is ordinarily used in subsequent coupling steps.

7) B. Helfrich, P. Schellenberg, and J. Ullrich, Chem. Ber., 90, 700 (1957).

8) G. W. Anderson, J. Blodinger, and A. D. Welcher, J. Amer. Chem. Soc., 74, 5309 (1952).

9) For discussion of electrophile-induced reactions of vinyl esters see: E. C. Leonard and M. K. Lindemann, pp 263-363 in Vinyl and Diene Monomers, Pt.1, Interscience, N.Y., 1970.

10) J. B. Hendrickson and C. Kandall, Tetrahedron Letters, 343 (1970).

11) For next in series see: R. A. Olofson, R. C. Schnur, L. Bunes, and J. P. Pepe, Tetrahedron Letters, Following communication.